October 2004

QIAamp® DSP 96 Virus MDx Kit Handbook

1152

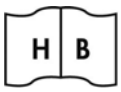
IVD

The BioRobot® MDx DSP system is a generic system that uses QIAamp technology for automated isolation and purification of viral nucleic acids from human specimens, such as plasma or serum samples, for in vitro diagnostic use.

For in vitro diagnostic use

CE

REF 61762



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Contents

Kit Contents

QIAamp DSP 96 Virus MDx Kit			
Catalog number			**61762**
Number of preps			**1152**
QIAamp DSP 96 Virus MDx Core Kit			**1 box**
QIAamp 96	QIAamp 96 Plate	PLATE	12
CARRIER	Carrier RNA (red cap)	CAR RNA	12 x 1350 µg
QP	QIAGEN® Protease*	QPROT	12 vials
PS	Protease Solvent	QPROT SOLV	12 x 6 ml
AL	Lysis Buffer†	LYS BUF	12 x 33 ml
AW1	Wash Buffer 1† (concentrate)	WASH BUF 1 CONC	4 x 151 ml
AW2	Wash Buffer 2‡ (concentrate)	WASH BUF 2 CONC	4 x 127 ml
AVE	Elution Buffer‡ (purple cap)	ELU BUF	108 x 2 ml
TOPE	Elution Fluid (orange cap)	ELU FLUID	48 x 1.4 ml
	Q-Card§		1
	Handbook	H B	1
QIAamp DSP 96 Virus MDx Plasticware			**1 box**
SB	S-Block	SBLOC	12
CAP	Caps for Strips	STRIP CAP	3 x 55
EMT	Elution Microtubes CL	ELU M TUBE	12
DT	Disposable Trough	TRO	3 x 10
BOT	Ethanol Bottle	EtOH BOT	1

* Resuspension volume 6 ml.

† Contains guanidine hydrochloride. See page 7 for safety information.

‡ Contains sodium azide as a preservative.

§ Do not discard the Q-Card. The information encoded in the bar code on the Q-Card is needed to start a run on the BioRobot MDx DSP system.

Symbols

	Content is sufficient for <N> tests
	Refer to information given in the handbook
	To be used by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number
COMP	Components
NUM	Number
VOL	Volume
	Temperature limitations
	Legal manufacturer
	Important note
	Write down the current date after adding ethanol to the bottle
ADD	Adding
CONT	Contains
EtOH	Absolute ethanol
GuHCl	Guanidine hydrochloride
LYOPH	Lyophilized
RCNS	Reconstitute in
SUBT	Subtilisin
USE	Only for use with
	Leads to

Storage

The QIAamp DSP 96 Virus MDx Kit must be stored dry at the temperature indicated on the kit label. The expiration date for the kit is printed on the Q-Card and on the kit label, and is valid only when the kit is stored at the temperature indicated on the kit label.

Carrier RNA can only be dissolved in elution buffer (AVE); dissolved carrier RNA must be immediately added to lysis buffer (AL) as described on page 26. This solution must be prepared fresh, and is stable at 2–8°C for several hours.

Reconstituted QIAGEN Protease (QP) is stable for up to 1 year when stored at 2–8°C, but only until the kit expiration date.

Reconstituted wash buffer 1 (AW1) and reconstituted wash buffer 2 (AW2) are stable for up to 1 year when stored at room temperature (15–25°C), but only until the kit expiration date.

Quality Control

In accordance with QIAGEN's certified Total Quality Management System, each lot of QIAamp DSP 96 Virus MDx Kit is tested against predetermined specifications to ensure consistent product quality.

Intended Use

The BioRobot MDx DSP system is a generic system that uses QIAamp technology for automated isolation and purification of viral nucleic acids from human specimens, such as plasma or serum samples, for in vitro diagnostic purposes. The BioRobot MDx DSP system is intended to be used only as a closed system. The system consists of the BioRobot MDx workstation, laboratory and accessory cabinets, a computer with QIAsoft MDx DSP software and QIAamp DSP 96 Virus MDx protocol, and the QIAamp DSP 96 Virus MDx Kit.

The BioRobot MDx DSP system is intended for use by professional users such as technicians and physicians trained in molecular biological techniques. Any in vitro diagnostic results generated using the sample preparation procedure in conjunction with any downstream in vitro diagnostic Nucleic Acid Testing (NAT) assay should be interpreted with regard to other clinical and laboratory findings.

To minimize the risk of a negative impact on in vitro diagnostic results, the user is instructed to use an internal control throughout the processes of sample preparation, amplification, and detection, according to the downstream assay used, in order to control for irregularities.

Product Use Limitations

The BioRobot MDx DSP system is not for isolation and purification of RNA and DNA from human whole blood samples, tissue, bone marrow, and cultured cells. It is not for use in isolation and purification of bacterial, fungal, and parasite nucleic acids.

Performance for sample materials such as CSF, urine, and fecal suspensions has not been established.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Lysis buffer (AL) and wash buffer 1 (AW1) contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid personal injury or injury to others.

QIAGEN has not tested the liquid waste generated by the QIAamp DSP 96 Virus MDx procedure for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

The following risk and safety phrases apply to the components of the QIAamp DSP 96 Virus MDx Kit.

Lysis buffer (AL) and wash buffer 1 (AW1)

 Xn

Contain guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

QIAGEN Protease (QP)

 Xn

Contains subtilisin (from *Bacillus subtilis*): sensitizer, irritant. Risk and safety phrases:* R37/38-41-42, S22-24-26-36/37/39-46

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R37/38: Irritating to respiratory system and skin; R41: Risk of serious damage to eyes; R42: May cause sensitization by inhalation; S13: Keep away from food, drink, and animal feedingstuffs; S22: Do not breathe dust; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S46: If swallowed, seek medical advice immediately and show this container or label.

Introduction

The QIAamp DSP 96 Virus MDx Kit uses well-established technology for simultaneous isolation and purification of viral DNA and RNA from a broad range of viruses. The kit combines the selective binding properties of a silica-based membrane with a high-throughput 96-well format, and is designed for fully automated, simultaneous processing of 96 samples on the BioRobot MDx DSP system. The fully automated process, including bar code reading, load check, sample tracking, and complete process documentation, requires a maximum of 2.5 hours to process 96 samples, with no hands-on time. Manual handling of potentially infectious samples is minimized, ensuring safety to the user and reliable processing of samples. Turnaround time between consecutive runs is approximately 25 minutes for reagent preparation and setup on the BioRobot MDx DSP workstation, plus 15 minutes for instrument cleanup.

The procedure is suitable for use with serum samples or with plasma samples containing either citrate or EDTA. Plasma samples containing heparin must not be used because this anticoagulant can interfere with downstream diagnostic assays, such as PCR. Samples can be fresh, lyophilized, or frozen, provided they have not been frozen and thawed more than once. Viral nucleic acids are eluted in elution buffer (AVE), ready for use in amplification reactions.

The QIAamp DSP 96 Virus MDx protocol provides the option of two different wash procedures. The standard QIAamp DSP 96 Virus MDx protocol uses wash procedures with wash buffer 2 (AW2) and ethanol (EtOH). The QIAamp DSP 96 Virus MDx (AW1) protocol provides a more stringent wash procedure with both wash buffers (AW1 and AW2) and ethanol (EtOH). This allows the flexibility to choose the desired purity for different downstream assays. The user needs to validate which protocol is best suited for a specific downstream assay, following guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) in *ICH Q2A: Validation of analytical methods: Definitions and terminology* and *ICH Q2B: Validation of analytical methods: Methodology*. Both protocols can be run on the same BioRobot MDx DSP system, as required.

Principle and procedure

The QIAamp DSP 96 Virus MDx procedure comprises 6 steps:

- Software-guided setup of the BioRobot MDx system, including bar code reading of samples
- Lysing the virus particles in the samples
- Binding the viral nucleic acids in the lysates to the membranes of a QIAamp 96 plate
- Washing the viral nucleic acids bound to the membranes
- Eluting the viral nucleic acids from the membranes
- Creation of result files

Sample volume

The BioRobot MDx DSP system is capable of removing a sample volume of 265 µl from primary and secondary tubes of various sizes, such as CryoTube® or BD Vacutainer® tubes, containing a minimum sample volume of 500 µl. Tables 1–3 (pages 10–11) list the tubes that are compatible with the different tube holders of the BioRobot MDx DSP. The BioRobot MDx DSP is not restricted to these tubes. If you prefer to use other tubes, the QIAGEN Instrument Service Specialist will test them during the installation of your BioRobot MDx DSP.

Note: Tubes with identical dimensions may be available under different catalog numbers, but with different anticoagulants.

Table 1. Compatible Tubes for Tube holder, STS MDx, 15 mm (holds tubes with inner diameter of 14–16 mm)

Manufacturer/ brand name	Volume	Height	Tube shape	Distributor	Cat. no.*
BD Falcon™	14 ml	96 mm	Round	BD	352051
BD Vacutainer	10 ml	100 mm	Round	BD	368430/ 366457
VENOJECT II	10 ml	100 mm	Round	Terumo	VP-100 SHL

* Catalog numbers are subject to change; please check with the manufacturer or supplier.

Table 2. Compatible Tubes for Tube holder, STS MDx, 12 mm (holds tubes with inner diameter of 11–13 mm)

Manufacturer/ brand name	Volume	Height	Tube shape	Distributor	Cat. no.†
Sarstedt	7 ml	81 mm	Round	Sarstedt	60550109
VENOJECT II	5 ml	78 mm	Round	Terumo	VP-050 SDK

† Catalog numbers are subject to change; please check with the manufacturer or supplier.

Table 3. Compatible Tubes for Tube holder, STS MDx, 9 mm (holds tubes with inner diameter of 8–10 mm)

Manufacturer/ brand name	Volume	Height	Tube shape	Distributor	Cat. no.*
BD Falcon	5 ml	75 mm	Round	BD	352063
KABE EDTA	5 ml	75 mm	Round	KABE	53101
NALGENE®	2 ml	45 mm	Cryogenic vial	Nalge	5000-0120
NUNC	1.8 ml	40 mm	Round	Nunc	363401
NUNC	3.6 ml	60 mm	Round	Nunc	366524
NUNC	4.5 ml	80 mm	Round	Nunc	363452
Sarstedt	2 ml	45 mm	Conical	Sarstedt	72693005
Sarstedt	5 ml	75 mm	Round	Sarstedt	62526028
BD Vacutainer	6 ml	75 mm	Round	BD	367815

* Catalog numbers are subject to change; please check with the manufacturer or supplier.

Lysing virus particles

Samples are lysed under denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Protease (QP) and lysis buffer (AL), which together ensure inactivation of RNases.

Binding nucleic acids to the membranes of the QIAamp 96 plate

To optimize the binding of viral DNA and RNA to the membranes of the QIAamp 96 plate, ethanol is first added to the lysates. Each lysate is then applied to a well of the QIAamp 96 plate, and viral nucleic acids are adsorbed

onto the silica-gel membranes as the lysates are drawn through by vacuum pressure.

Removing residual contaminants

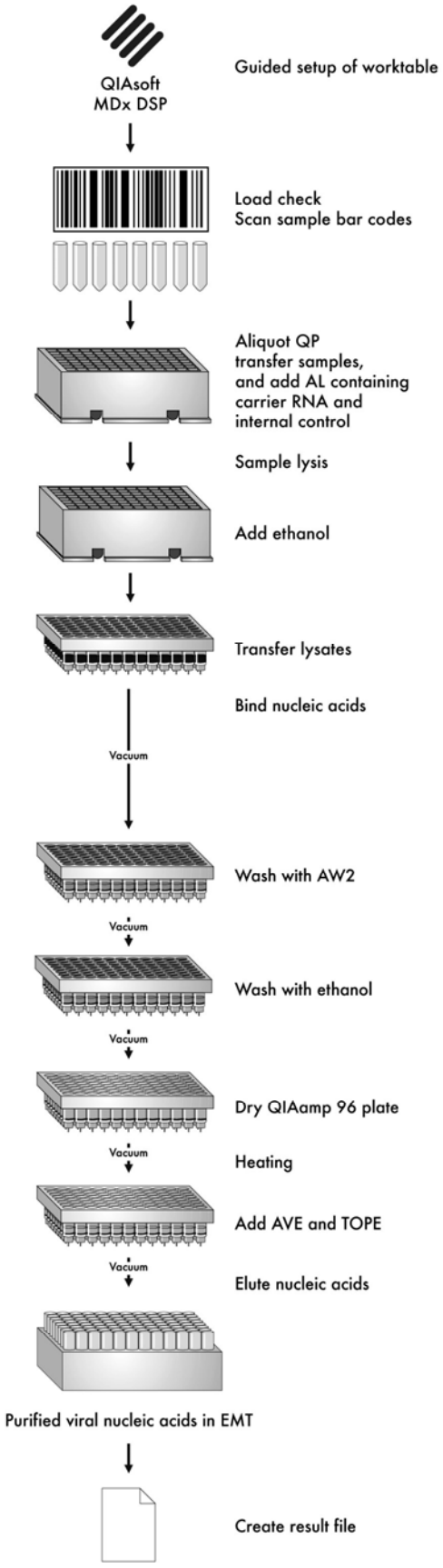
While the viral nucleic acids remain bound to the membranes of the QIAamp 96 plate, contaminants are efficiently washed away, in the standard QIAamp DSP 96 Virus MDx protocol, using first wash buffer 2 (AW2), and then ethanol (EtOH).

Alternatively, the QIAamp DSP 96 Virus MDx (AW1) protocol provides a more stringent wash. This protocol differs from the standard protocol by the addition of a wash step with wash buffer 1 (AW1) before washing with wash buffer 2 (AW2), and then with ethanol (EtOH).

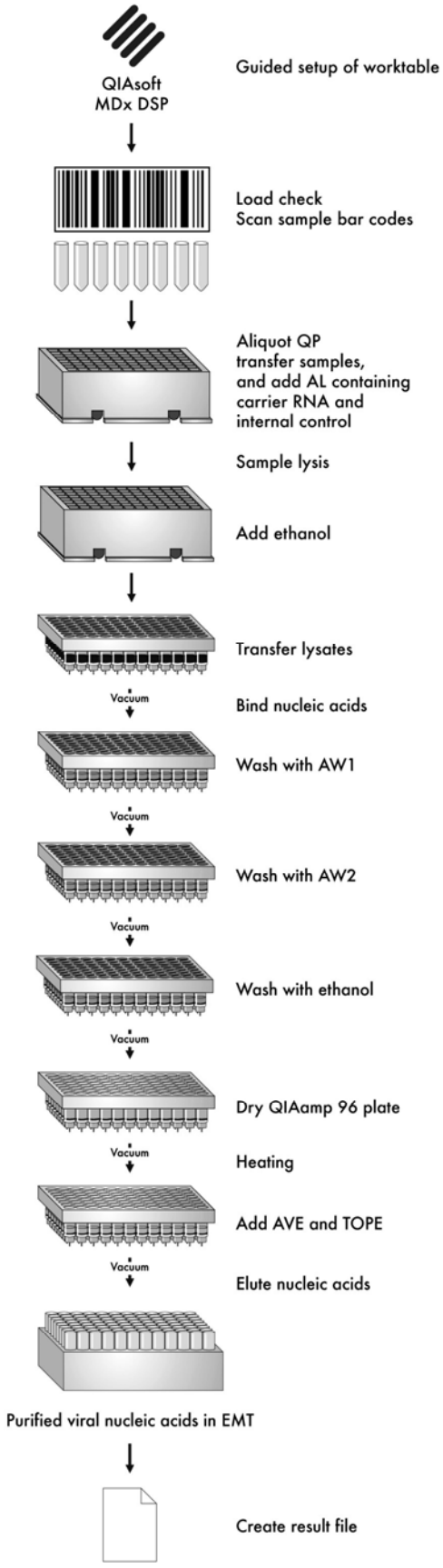
Eluting pure nucleic acids

Viral nucleic acids are eluted from the membranes of the QIAamp 96 plate using elution buffer (AVE). Depending on the downstream assay used, each nucleic acid eluate can comprise up to 50% of the reaction volume without any inhibitory effects.

QIAamp DSP 96 Virus MDx Protocol



QIAamp DSP 96 Virus MDx (AW1) Protocol



Performance characteristics of the BioRobot MDx DSP system

Linear range

The linear range for both QIAamp DSP 96 Virus protocols was evaluated for HIV and HCV RNA viruses, and HBV DNA virus. The tests were performed with dilutions of calibrated virus panels made in HIV-, HBV-, and HCV-negative human plasma. Quantification of HIV and HBV virus panels was performed using WHO International Standards 97/656 and 97/746, respectively, as reference material. A commercially available, calibrated virus panel from AcroMetrix was used for the HCV dilution series. Dilution series with 7 different virus titers were tested with 24 replicates each.

The linear range of the QIAamp DSP 96 Virus MDx procedure has been determined for HIV and HCV RNA viruses, and HBV DNA virus, in several downstream diagnostic assays (Table 4 and Figures 1–3). Calculation of titers (conversion from cp to IU values) were performed according to the specifications of the corresponding downstream assay manufacturer (Table 4).

Table 4. Downstream Diagnostic Assays Used for Determination of Linear Range of Yields with QIAamp DSP 96 Virus MDx Protocols

Virus	Downstream assay	Assay manual used	Conversion factor
HIV	LCx® HIV RNA Quantitative Assay (ABBOTT Laboratories)	12/2003	1 IU = 0.1412 cp
HBV	COBAS AMPLICOR® HBV MONITOR® Test (Roche Diagnostics GmbH)	7/2003, Revision 1.0	1 IU = 5.26 cp
HCV	COBAS TaqMan HCV Test (Roche Diagnostics GmbH)	9/2003	1 IU = 1 cp

Linear Range of Yields Using QIAamp DSP 96 Virus MDx Protocols — HIV

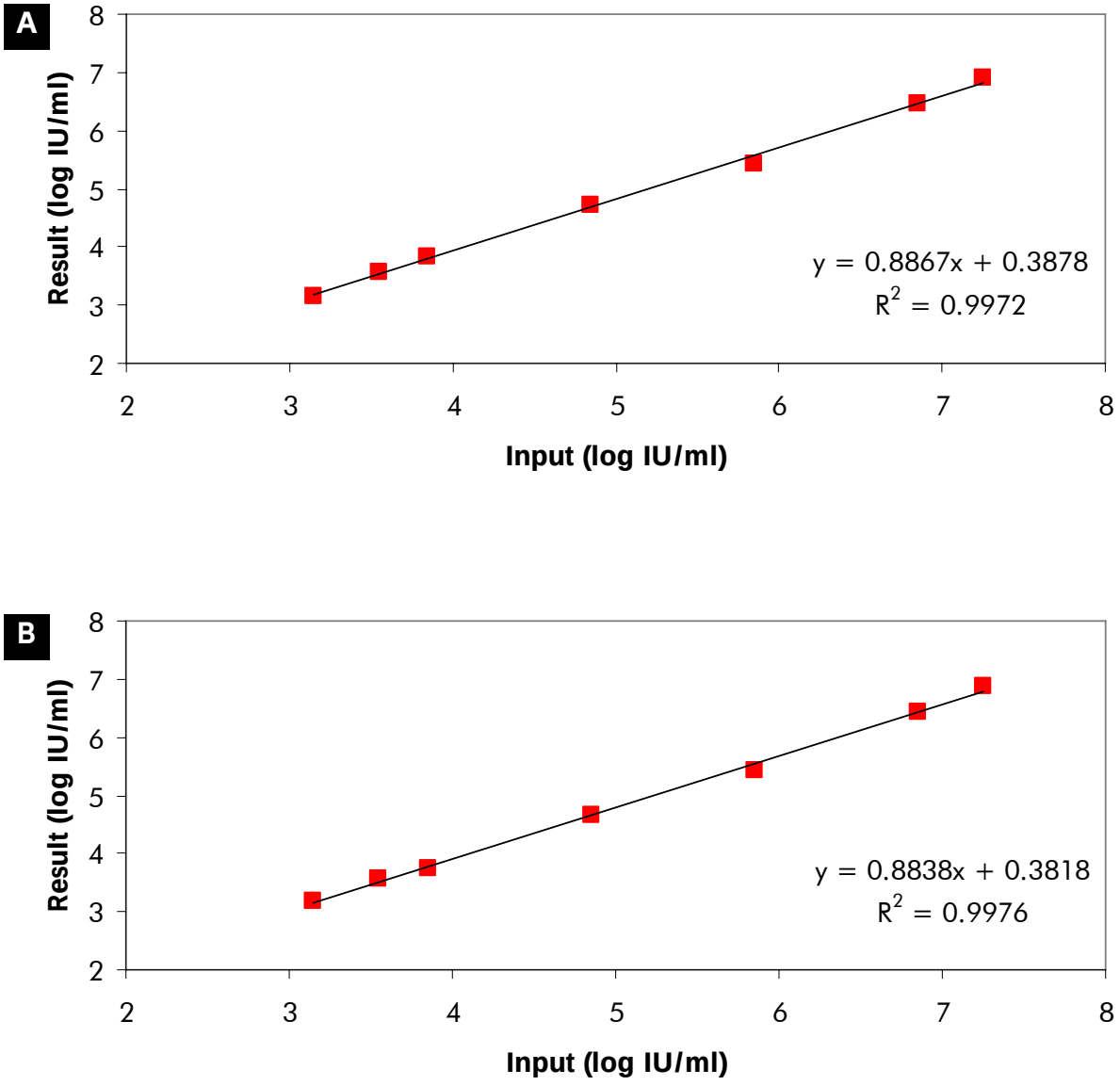


Figure 1 The linear range of the QIAamp DSP 96 Virus MDx protocols was determined using the LCx HIV RNA Quantitative Assay **A** with the QIAamp DSP 96 Virus MDx (AW1) protocol and **B** with the standard QIAamp DSP 96 Virus MDx protocol. IU values were calculated using the specifications given in the manual for the downstream assay (1 IU = 0.1412 cp).

Linear Range of Yields Using the QIAamp DSP 96 Virus MDx Protocol — HCV

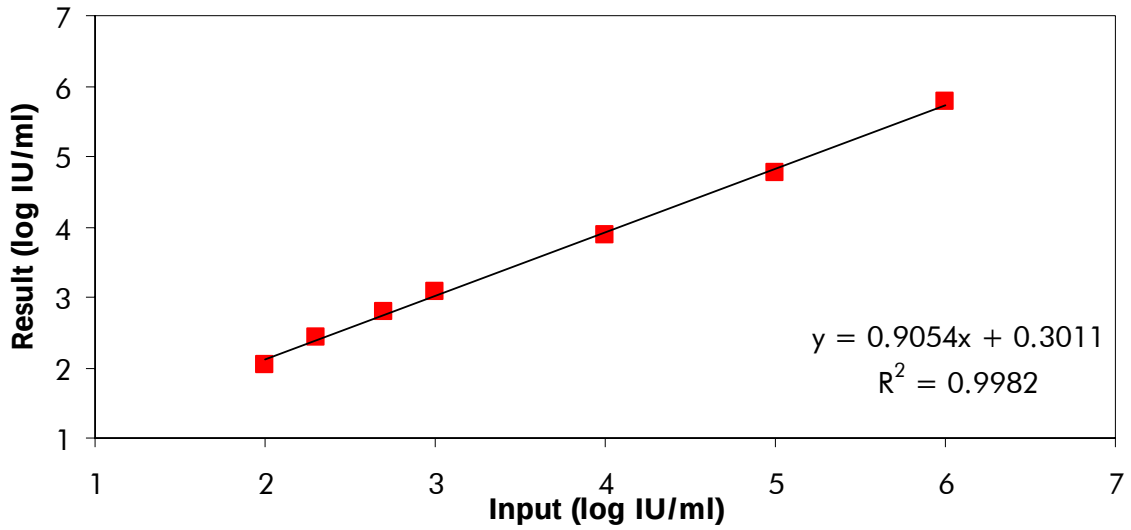


Figure 2 The linear range of the QIAamp DSP 96 Virus MDx protocol was determined using the COBAS TaqMan HCV Test. IU values were calculated using the specifications given in the manual for the downstream assay (1 IU = 1 cp).

Linear Range of Yields Using the QIAamp DSP 96 Virus MDx (AW1) Protocol — HBV

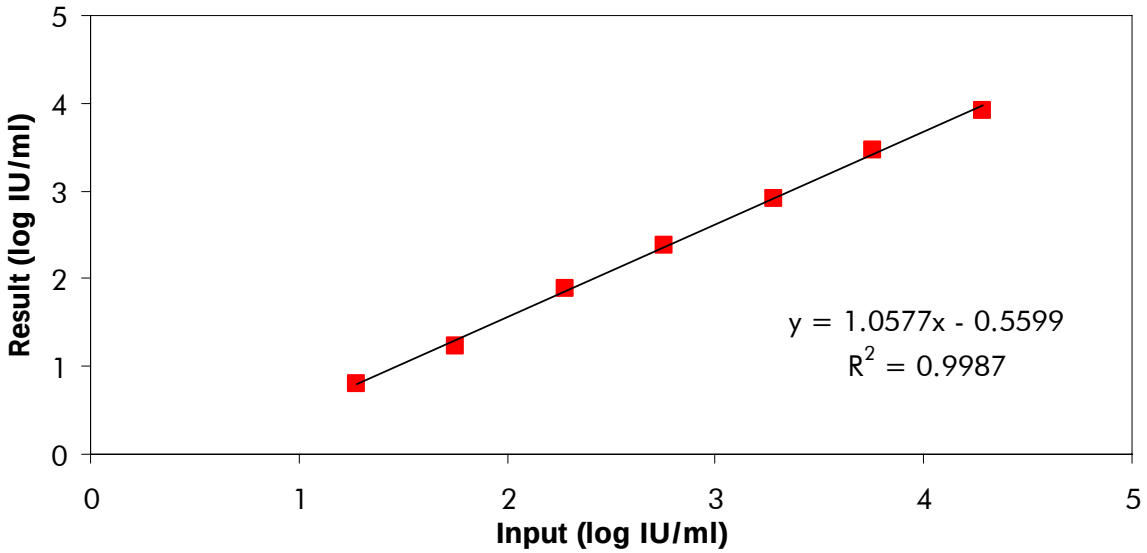


Figure 3 The linear range of the QIAamp DSP 96 Virus MDx (AW1) protocol was determined using the COBAS AMPLICOR HBV MONITOR Test. IU values were calculated using the specifications given in the manual for the downstream assay (1 IU = 5.26 cp).

Precision

Coefficients of variation (CVs) were determined for HIV, HCV, and HBV dilution series in the linear range of the appropriate downstream assay. For precision analysis, the same downstream assays, dilution series, and data sets were used as for determination of the linear range. Calculation of titers (conversion from cp to IU values) were performed according to the specifications of the corresponding downstream assay manufacturer (see Table 4, page 14). The intra-run, inter-run, and total precision data are shown in percent coefficient of variation (%CV) in Tables 5–8.

Table 5. Precision of the QIAamp DSP 96 Virus MDx (AW1) Protocol in the LCx HIV RNA Quantitative Assay

Mean titer (IU/ml)	Intra-run precision (%CV)	Inter-run precision (%CV)	Total precision (%CV)
8.01×10^6	9.38	5.03	10.64
2.86×10^6	13.62	0.00	13.62
2.63×10^5	25.09	10.28	27.11
5.09×10^4	11.38	5.50	12.63
7.00×10^3	19.93	15.77	25.41
3.71×10^3	32.41	0.00	32.41
1.44×10^3	78.10	0.00	78.10

Table 6. Precision of the QIAamp DSP 96 Virus MDx Protocol in the LCx HIV RNA Quantitative Assay

Mean titer (IU/ml)	Intra-run precision (%CV)	Inter-run precision (%CV)	Total precision (%CV)
7.49×10^6	14.37	3.89	14.89
2.74×10^6	11.06	5.47	12.34
2.61×10^5	12.45	0.00	12.45
4.71×10^4	12.92	0.00	12.92
5.64×10^3	26.98	0.00	26.98
3.69×10^3	45.35	0.00	45.35
1.56×10^3	74.41	11.40	75.28

Table 7. Precision of the QIAamp DSP 96 Virus MDx Protocol in the COBAS TaqMan HCV Test

Mean titer (IU/ml)	Intra-run precision (%CV)	Inter-run precision (%CV)	Total precision (%CV)
5.92×10^5	10.31	11.05	15.12
6.01×10^4	16.24	4.09	16.75
7.47×10^3	16.46	8.62	18.58
1.16×10^3	17.73	0.00	17.73
6.13×10^2	25.15	0.00	25.15
2.74×10^2	35.74	28.56	45.75
1.07×10^2	39.97	0.00	39.97

Table 8. Precision of the QIAamp DSP 96 Virus MDx (AW1) Protocol in the COBAS AMPLICOR HBV MONITOR Test

Mean titer (IU/ml)	Intra-run precision (%CV)	Inter-run precision (%CV)	Total precision (%CV)
8.30×10^3	11.24	23.79	26.31
2.85×10^3	24.52	24.91	34.95
8.04×10^2	19.08	25.84	32.12
2.39×10^2	20.51	24.98	32.32
7.77×10^1	15.02	25.28	29.40
1.71×10^1	53.02	0.00	53.02
6.34×10^0	47.56	48.59	67.99

Detection limit

The detection limit was determined by the 95% probit value for both QIAamp DSP 96 Virus MDx protocols using HIV and HBV WHO international virus standards 97/656 and 97/746, respectively. The detection limit was determined by processing dilution series of the appropriate viruses (WHO international virus standard diluted in HIV- and HBV-negative normal human plasma with citrate). Each dilution step was tested in 3 runs on the BioRobot MDx DSP system giving 36 replicates per dilution. An in-house, real-time RT-PCR assay was used for detection of HIV RNA. HBV DNA was analyzed using an in-house, endpoint PCR method, followed by agarose gel electrophoresis. Amplicons of the expected size were counted as hits. The combined data for all samples were evaluated using probit analysis (Logistic regression, Arcus Quickstat, Biomedical Version 1.1). The data are presented in Tables 9 and 10.

Table 9. Detection Limit of HIV RNA Using QIAamp DSP 96 Virus MDx Protocols and an In-House, Real-Time RT-PCR Assay

		QIAamp DSP 96 Virus MDx (AW1) protocol		QIAamp DSP 96 Virus MDx protocol	
Input titer (IU/ml)	**Replicates**	**Hits**	**Hits (%)**	**Hits**	**Hits (%)**
800	36	36	100.00	36	100.00
600	36	36	100.00	36	100.00
400	36	36	100.00	36	100.00
200	36	36	100.00	35	97.22
100	36	35	97.22	33	91.67
50	36	23	63.89	27	75.00
10	36	6	16.67	7	19.44
0	18	0	0.00	0	0.00
95% probit value	–	123.08	–	133.92	–
Confidence interval	–	87.84–205.19	–	92.38–234.94	–

Table 10. Detection Limit of HBV RNA Using QIAamp DSP 96 Virus MDx Protocols and an In-House PCR Assay

		QIAamp DSP 96 Virus MDx (AW1) protocol		QIAamp DSP 96 Virus MDx protocol	
Input titer (IU/ml)	**Replicates**	**Hits**	**Hits (%)**	**Hits**	**Hits (%)**
50	36	36	100.00	36	100.00
35	36	33	91.67	36	100.00
20	36	31	86.11	33	91.67
10	36	27	75.00	23	63.89
5	36	11	30.56	19	52.78
2	36	9	25.00	11	30.56
0.5	36	4	11.11	4	11.11
0	18	0	0.00	0	0.00
95% probit value	–	51.73	–	37.51	–
Confidence interval	–	33.08–100.01	–	24.27–72.53	–

System robustness

Results were compared for plasma and serum samples from individual donors collected using different blood drawing tubes (Table 11) and spiked with HIV and HCV virus standards. The samples were processed using the QIAamp DSP 96 Virus MDx protocols (Figures 4 and 5).

Table 11. Combinations of Tubes and Viruses Spiked into Plasma Samples for Determination of System Variation

Virus	Tube	Abbreviation	Supplier	Cat. no.*
HIV	Vacutainer PPT™	PPT	BD	362788
HIV	Vacuette® EDTA	Vacuette	Greiner bio-one	454036
HIV	Primavette S EDTA	Primavette	Kabe Labortechnik	0959 0540
HCV	Vacutainer PPT	PPT	BD	362788
HCV	Monovette® Coagulation 9 NC	S 9NC	Sarstedt	02.1067.001
HCV	Monovette Serum Gel S 9	S Gel	Sarstedt	02.1388.001

* Catalog numbers are subject to change; please check with the manufacturer or supplier.

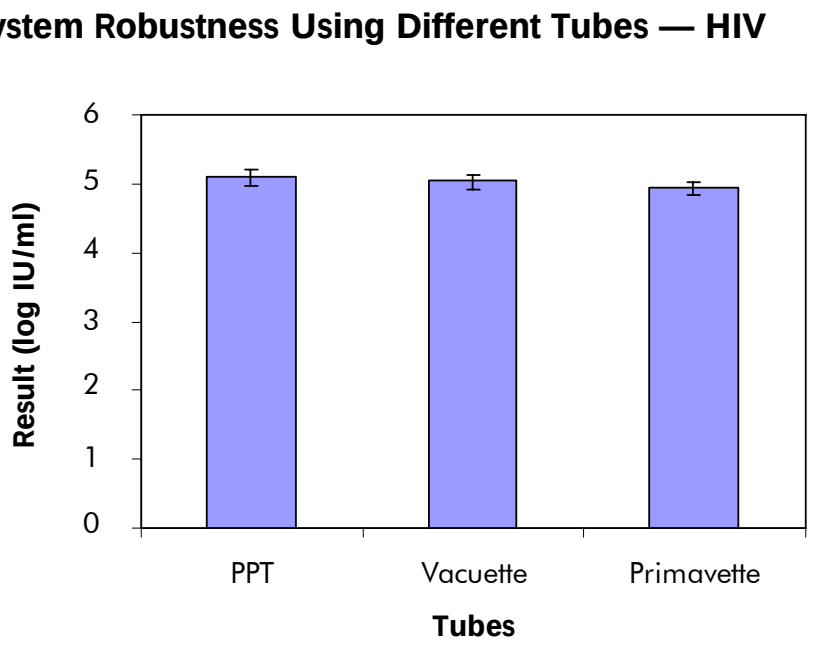


Figure 4 An HIV in-house virus working standard, calibrated to the WHO International standard, was used to spike plasma samples with HIV at a titer of 1.4 x 10^5 IU/ml. HIV RNA was purified using the QIAamp DSP 96 Virus MDx protocol and detected using the COBAS TaqMan HIV-1 Test (Roche Diagnostics GmbH). The tubes used are listed in Table 11. IU values were calculated using the specifications given in the manual for the downstream assay (1 IU = 0.61 cp).

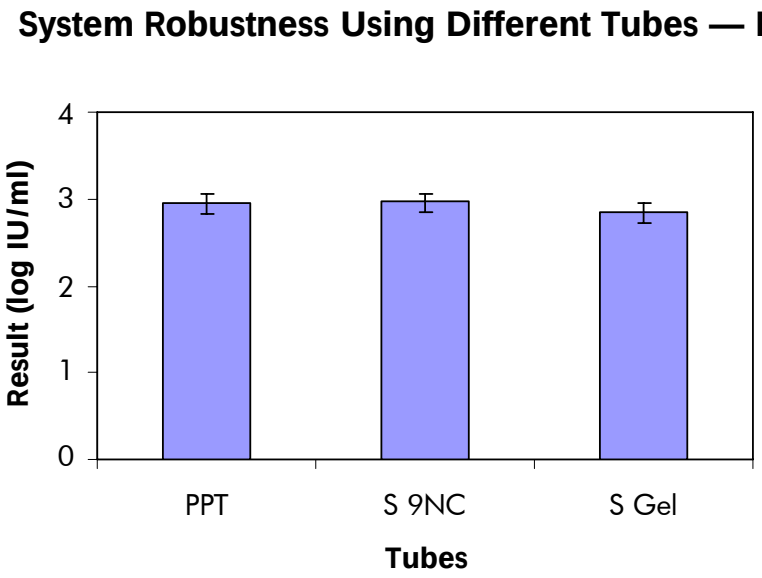


Figure 5 Commercially available HCV virus standard from AcroMetrix was used to spike donor samples to a titer of 500 IU/ml. For detection, the COBAS TaqMan HCV Test (Roche Diagnostics GmbH) was used as the downstream diagnostic assay. Sample preparation was performed using the QIAamp DSP 96 Virus MDx (AW1) protocol. The tubes used are listed in Table 11, page 22. IU values were calculated using the specifications given in the manual for the downstream assay (1 IU = 1 cp).

Exclusion of sample carryover

Six runs on the BioRobot MDx DSP system were performed to evaluate the risk of cross-contamination events during and between the QIAamp DSP 96 Virus MDx protocols. The test was performed using a commercially available, quantified HBV virus standard from AcroMetrix. The viral load of positive samples used for the carryover tests was 2.0×10^5 IU/ml. For dilution of positive samples and as negative control samples, human HBV-negative plasma with citrate was used.

To detect sample-to-sample carryover, 3 runs were performed with a checkerboard setup of negative and highly positive samples. After each checkerboard run, one run with all negative samples was performed to monitor possible run-to-run carryover. HBV DNA was detected with a validated, in-house, endpoint PCR assay, followed by agarose gel electrophoresis. The assay has been validated to be capable of detecting HBV DNA with a 95% probability at a virus titer of 16.76 IU/ml (95% probit).

All of the highly positive samples were detected positive using the in-house HBV PCR assay. All negative samples, in the checkerboard runs and the all-negative runs, were nonresponsive.

These experiments demonstrate that the QIAamp DSP 96 Virus MDx protocols provide no sample carryover under these conditions.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- BioRobot MDx DSP system, including BioRobot MDx workstation, QIAsoft MDx DSP software, and QIAamp DSP 96 Virus MDx protocol
- Disposable Filter-Tips, 1100 µl (960) (cat. no. 9012598)
- Ethanol (96–100%, purity grade p.a.)
- Deionized distilled water
- Disposable gloves
- Measuring cylinders (200, 500, and 1000 ml)
- Centrifuge with compatible rotor for centrifuging sample tubes
- Pipets and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipet tips with aerosol barriers)

Important Notes

Important general points

- After receiving the kit, check the kit components for damage. If the blister packs or the bottles are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (page 7). Do not use damaged kit components, since their use may lead to poor kit performance.
- Always use RNase-free equipment.
- Always change pipet tips between manual transfer of liquids (e.g., when preparing, pooling, or aliquoting viral samples to be placed on the BioRobot MDx worktable).
- Always use disposable filter-tips from QIAGEN (see page 24) to avoid cross-contamination.
- Always use disposable gloves and regularly check that they are not contaminated with sample material.
- Discard gloves if they become contaminated.
- To ensure safety from potentially infectious material, we recommend working under laminar air-flow conditions until the samples are placed on the BioRobot MDx worktable.
- Do not use kit components from another QIAamp DSP 96 Virus MDx Kit, unless the material numbers and the lot numbers of the two kits are identical.
- Do not pool reagents from different bottles or tubes, even if they are from the same lot or the same kit.
- After use, close all bottles in order to avoid leakage.
- Do not use a kit or kit component after its expiration date.
- Avoid microbial contamination of the kit reagents.
- This system should only be used by personnel trained in in vitro diagnostic laboratory practice and in operating the BioRobot MDx DSP system. Personnel should also refer to the *BioRobot MDx DSP User Manual*.

Preparing RNA

Elution buffer (AVE) is tested for the absence of detectable levels of RNases and contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase-degrading chemicals, it will not actively inhibit RNases introduced by

inappropriate handling. Extreme care should be taken to avoid contamination with RNases when handling elution buffer (AVE).

Storing samples

After collection and preparation, plasma or serum samples can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –20°C or –80°C in aliquots is recommended. Frozen plasma or serum samples must not be thawed more than once. Repeated freeze–thawing may lead to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitates formed during freeze–thawing may clog the membranes of the QIAamp 96 plate. If cryoprecipitates are visible, they should be pelleted by centrifugation at approximately 6800 x *g* for 3 minutes. The cleared supernatant should be aspirated and processed immediately without disturbing the pellet. Before starting the viral nucleic acid purification procedure, thawed samples must be equilibrated to room temperature (15–25°C).

Preparing reagents and buffers

Preparing QIAGEN Protease

Add the entire contents of one bottle containing 6 ml protease solvent (PS) to one vial of lyophilized QIAGEN Protease (QP) and mix carefully. To avoid foaming, mix by inverting the vial several times. Ensure that the QIAGEN Protease (QP) is completely dissolved. Open the vial and place it in the reagent holder for 1 bottle (QP) on the BioRobot MDx worktable.

Adding carrier RNA to lysis buffer

Carrier RNA serves 2 purposes. Firstly, it enhances binding of viral nucleic acids to the membranes of the QIAamp 96 plate, especially if there are very few target molecules in the samples. Secondly, the addition of large amounts of carrier RNA reduces the chance of viral RNA degradation in the rare event that RNase molecules are not denatured by the chaotropic salts and detergent in lysis buffer (AL). If carrier RNA is not added to lysis buffer (AL), this may lead to reduced viral RNA or DNA recovery.

Carrier RNA may also be included in some internal control reagents of commercial downstream assays. In these cases, please refer to the relevant instructions for use from the manufacturer of the downstream assay.

To prepare the carrier RNA solution, add 450 µl elution buffer (AVE) to one vial containing 1350 µg lyophilized carrier RNA. One tube of elution buffer (AVE) is provided for each vial of carrier RNA.

Note that carrier RNA does not dissolve in lysis buffer (AL). It must first be dissolved in elution buffer (AVE) and then added to lysis buffer (AL). Ensure that the carrier RNA is completely dissolved in the correct volume of elution buffer (AVE) before mixing it with lysis buffer (AL).

Transfer 230 µl of carrier RNA reconstituted in elution buffer (AVE) to one bottle containing 33 ml lysis buffer (AL) to give a final concentration of 5.6 µg carrier RNA per sample preparation. Gently mix by inverting the bottle 10 times. To avoid foaming, do not vortex. Pour the entire lysis buffer (AL)/carrier RNA mix into a disposable trough (DT) in the reagent holder for 2 troughs (position AL) and remove any large air bubbles by touching them with a pipet tip.

ⓘ Carefully pour lysis buffer (AL) containing carrier RNA into the disposable trough (DT) in order to avoid creating large bubbles, which may interfere with the liquid detection system.

Adding internal control to lysis buffer

Use of an internal control is strongly recommended when using the QIAamp DSP 96 Virus MDx Kit in combination with diagnostic amplification and detection systems. Internal control RNA or DNA and reconstituted carrier RNA should be added to lysis buffer (AL), and mixed thoroughly by inverting the bottle 10 times. To avoid foaming, do not vortex.

Refer to the manufacturer's instructions to determine the optimal concentration of internal control. Using a concentration other than that recommended may result in incorrect results. When calculating the correct amount of internal control to use, take into consideration the starting volume of the sample and the volume of the eluate. For your calculations, use a starting sample volume of 265 µl and an eluate volume of 83 µl (see "Eluting viral nucleic acids", page 31). See Appendix C for an example of how to calculate the amount of internal control to use with the BioRobot MDx DSP system and diagnostic downstream assays.

ⓘ Always use the correct internal control for the downstream assay. Refer to manufacturers' instructions for further information. See also Appendix C for an example of how to calculate the amount of internal control to use with the BioRobot MDx DSP system.

ⓘ If an internal control is added to the lysis buffer (AL) and carrier RNA, make sure to transfer no more than 33 ml of the mixture into the disposable trough (DT). Discard the remainder. Using larger volumes may lead to incorrect liquid measurement with the liquid level detection system, resulting in failure of the load check procedure.

Preparing wash buffer 1 (for the QIAamp DSP 96 Virus MDx (AW1) protocol only)

Using a measuring cylinder, add 200 ml ethanol (96–100%) to a bottle containing 151 ml wash buffer 1 (AW1) concentrate. One bottle of reconstituted wash buffer 1 (AW1) contains enough buffer for 3 runs of 96 samples each. Store the reconstituted wash buffer 1 (AW1) at room temperature (15–25°C). To enable identification by the BioRobot MDx DSP system, the wash buffer 1 (AW1) bottle has a bar code on its label. The opened bottle should be placed in the reagent carousel with the bar code facing outward. This reagent is only required for the QIAamp DSP 96 Virus MDx (AW1) protocol. For the standard QIAamp DSP 96 Virus MDx protocol, the wash buffer 1 (AW1) bottle can either be removed from the reagent rotor or left in place.

ⓘ Always mix the reconstituted wash buffer 1 (AW1) by inverting the bottle several times before placing the bottle in the reagent carousel.

ⓘ After a run, close the bottle if another run will not be carried out immediately afterwards. Remove the cap when starting another run.

Preparing wash buffer 2

Using a measuring cylinder, add 300 ml ethanol (96–100%) to a bottle containing 127 ml wash buffer 2 (AW2) concentrate. One bottle of reconstituted wash buffer 2 (AW2) contains enough buffer for 3 runs of 96 samples each. Store the reconstituted wash buffer 2 (AW2) at room temperature (15–25°C). To enable identification by the BioRobot MDx DSP system, the wash buffer 2 (AW2) bottle has a bar code on its label. The opened bottle should be placed in the reagent carousel with the bar code facing outward.

ⓘ Always mix the reconstituted wash buffer 2 (AW2) by inverting the bottle several times before placing the bottle in the reagent carousel.

ⓘ After a run, close the bottle if another run will not be carried out immediately afterwards. Remove the cap when starting another run.

Preparing ethanol

Fill the empty ethanol bottle (BOT) with 350 ml ethanol (96–100%). Add 350 µl of reconstituted wash buffer 2 (AW2) to this bottle (for a final 1/1000 dilution of AW2); this allows the BioRobot MDx DSP system to detect the fill level in the bottle by conductivity measurements. The filled bottle contains enough ethanol for 2 runs of 96 samples each. Store the bottle at room temperature (15–25°C). To enable identification by the BioRobot MDx DSP system, the ethanol bottle (BOT) has a bar code on its label. The opened bottle should be placed in the reagent carousel with the bar code facing outward.

ⓘ Always mix the ethanol by inverting the bottle several times before placing the bottle in the reagent carousel.

ⓘ After a run, close the bottle if another run will not be carried out immediately afterwards. Remove the cap when starting another run.

ⓘ Make sure that 350 µl of reconstituted wash buffer 2 (AW2) is added to 350 ml ethanol (EtOH), as described above, in order to enable measurement of conductivity by the BioRobot MDx DSP system.

Preparing system liquid

Fill the empty system liquid bottle in the reagent rotor with at least 700 ml deionized water. The filled bottle contains enough system liquid for 1 run of 96 samples and the cleanup procedure after the run. The opened bottle should be placed in its matching slot in the reagent carousel.

Preparing elution buffer

For 1 run of 96 samples, 8 tubes of elution buffer (AVE) are required. Open these tubes and place them in the reagent holder for microtubes and trough (position AVE). Take care not to contaminate the buffers with RNases. Discard any elution buffer (AVE) left over after a run.

Preparing elution fluid

For 1 run of 96 samples, 4 tubes of elution fluid (TOPE) are required. Open these tubes and place them in the reagent holder for microtubes and trough (position TOPE). Discard any elution fluid (TOPE) left over after a run.

Preparing plasticware

S-Blocks

For each run, 1 S-Block (SB) must be placed onto the silver heat transfer adapter of the cooling and heating system. Ensure that position A1 is located at the upper-left corner. Discard the S-Block after the run.

Disposable troughs

For each run, 2 disposable troughs (DT) must be placed in the reagent holder for 2 troughs (positions AL and EtOH) on MP Slot 2. Discard the disposable troughs (DT) after the run.

Elution Microtubes CL

For each run, 1 Elution Microtubes CL (EMT) must be placed onto the blue elution microtube adapter. The assembly of Elution Microtubes CL (EMT) and adapter must be placed on top of the reagent holder for microtubes and trough. Ensure that the bar code of the Elution Microtubes CL (EMT) faces to the right.

QIAamp 96 plates

For each run, a QIAamp 96 plate must be placed in the silver multiwell-plate holder. Ensure that position A1 is located at the upper-left corner and the bar code of the QIAamp 96 plate faces to the right. Discard the QIAamp 96 plate after the run.

Preparing large liquid containers

When you start the QIAamp DSP 96 Virus MDx protocol on the BioRobot MDx DSP system, display messages will appear and ask you to prepare the system liquid container, waste container, and vacuum trap.

System liquid container

Empty the system liquid container (from underneath the workstation). When opening the system liquid container, first disconnect the tubing, then remove the lid. Rinse the container thoroughly with fresh deionized water and refill it with fresh deionized water. After refilling the system liquid container with deionized water, first close the lid, then connect the tubing. Flush the dilutor system with 30 ml deionized water by selecting "Tools/Flush System".

Waste container and vacuum trap

Empty the vacuum trap and the waste container (from underneath the workstation). When opening the vacuum trap, first disconnect the tubing, then remove the lid. After emptying the vacuum trap, first close the lid, then connect the tubing. The trap and container, and their lids and tubing, may be cleaned by washing with detergent and then rinsing with water.

ⓘ The liquid waste generated by QIAamp DSP 96 Virus MDx procedures has not been tested for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely but cannot be completely excluded. Therefore, liquid waste must be considered infectious and must be handled and discarded according to local safety regulations.

Eluting viral nucleic acids

Eluted viral nucleic acids are collected in Elution Microtubes CL (EMT). The mean volume of elution buffer (AVE) added by the BioRobot MDx DSP system per well of the QIAamp 96 plate is 91 µl. The mean volume of each eluate collected in the Elution Microtubes CL (EMT) is 83 µl.

Yield and quality of viral nucleic acids

The yield and quality of the isolated viral nucleic acids are suitable for all types of downstream detection procedure in molecular diagnostics. The yield and purity of the eluted viral nucleic acids may vary, depending on whether the protocol with or without wash buffer 1 (AW1) was used. The user needs to validate which protocol is best suited for a specific downstream assay, following ICH guidelines in *ICH Q2A: Validation of analytical methods: Definitions and terminology* and *ICH Q2B: Validation of analytical methods: Methodology*. Diagnostic assays should be performed according to the manufacturers' instructions.

Setting up the BioRobot MDx workstation

After you open the QIAsoft MDx DSP software and start one of the sample-preparation protocols (see protocol, page 36), a series of display messages will appear and guide you through setting up the BioRobot MDx workstation for the automated viral nucleic acid purification procedure. It is important to follow each display message before proceeding to the next one. Figure 6 (page 32) and Tables 12 and 13 (pages 10 and 11) summarize the layout of the BioRobot MDx worktable and the locations in which items must be loaded.

Cleaning up the BioRobot MDx workstation

At the end of a protocol run, the QIAsoft MDx DSP software displays a series of messages that guides you through the steps of the cleanup procedure. After each run, always discard the plasticware and reagents that remain on the worktable (AL, AVE, TOPE, and ethanol) in order to avoid carryover of viral nucleic acids to the next protocol run. The reagents left over in the reagent carousel (AW1 and AW2) can be used in several protocol runs until the expiration date specified on the bottle labels.

ⓘ Do not pool reagents from different bottles or tubes, even if they are from the same lot or the same kit.

ⓘ The liquid waste generated by the QIAamp DSP 96 Virus MDx procedure has not been tested for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely but cannot be

completely excluded. Therefore, liquid wasted must be considered infectious and must be handled and discarded according to local safety regulations.

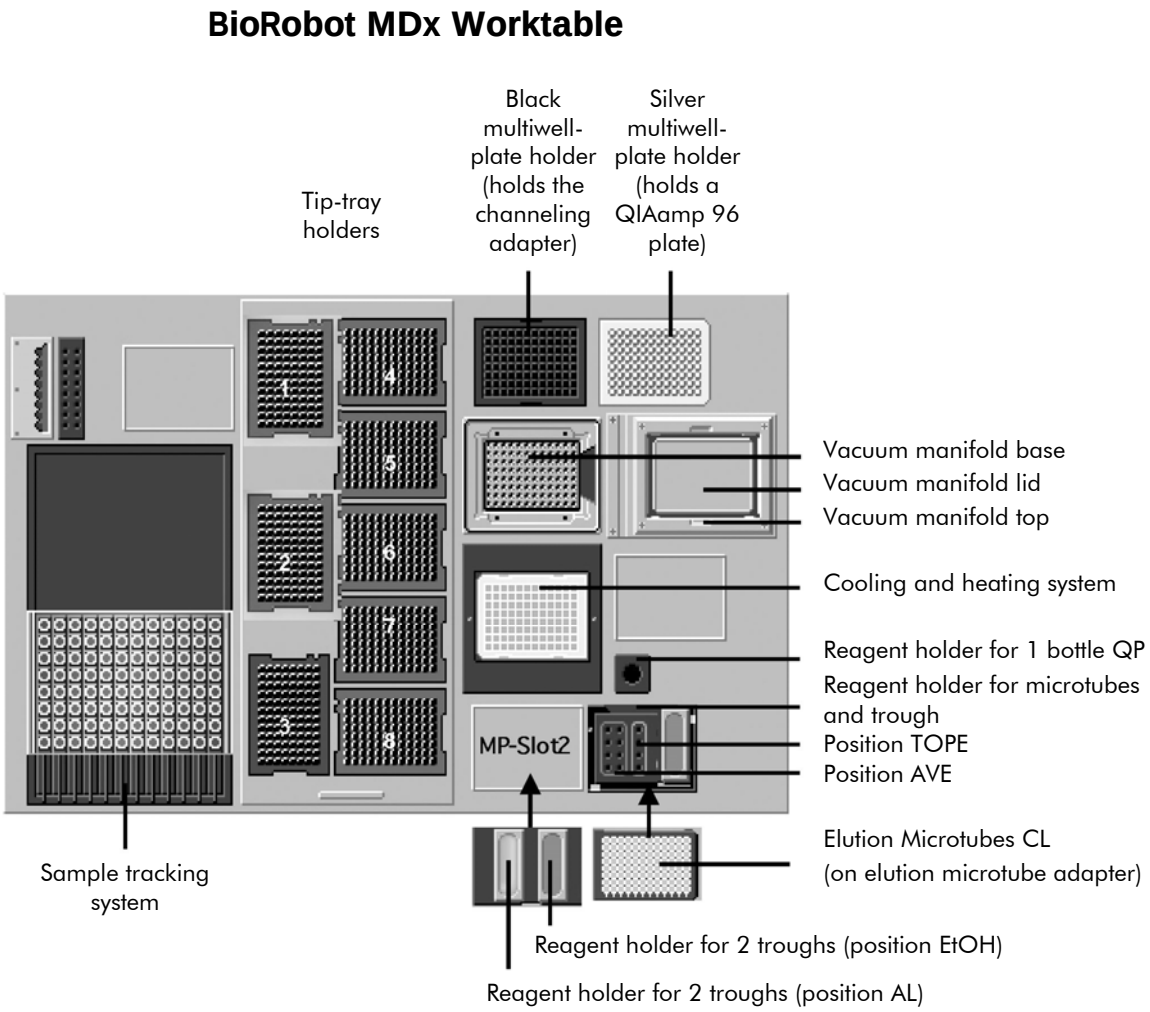


Figure 6 Overview of the BioRobot MDx worktable and its main components. Refer to Tables 12 and 13 for more details.

Table 12. Loading Plasticware and Accessories

Item	Position	Holder/adapter
Elution Microtubes CL (EMT)	Reagent holder for microtubes and trough	Blue elution microtube adapter
Disposable trough (DT)	MP Slot 2	Reagent holder for 2 troughs (EtOH and AL)
QIAamp 96 plate	Silver multiwell-plate holder	
Channeling adapter	Black multiwell-plate holder	
S-Block (SB)	Cooling and heating system	Silver heat transfer adapter
Disposable tips (1100 µl)	Red tip-tray holders	
Vacuum manifold lid	To the right of the vacuum manifold base	
Vacuum manifold top	To the right of the vacuum manifold base, and over the vacuum manifold lid	

Table 13. Loading Buffers and Reagents

Item	Position	Volume
Lysis buffer (AL)*	Disposable trough (DT) in reagent holder for 2 troughs (position AL)	33 ml
QIAGEN Protease (QP)†	Reagent holder for 1 bottle (QP)	6 ml
Elution buffer (AVE)	Reagent holder for microtubes and trough (position AVE)	Eight 2 ml tubes (purple caps)
Elution fluid (TOPE)‡	Reagent holder for microtubes and trough (position TOPE)	Four 1.5 ml tubes (orange caps)
Wash buffer 1 (AW1)§	Reagent carousel	Minimum of 164 ml (reconstituted)
Wash buffer 2 (AW2)	Reagent carousel	Minimum of 191 ml (reconstituted)
Ethanol	Reagent carousel	Minimum of 350 ml
System liquid	Reagent carousel	Minimum of 700 ml deionized water
Samples	Sample tracking system	Minimum of 500 µl sample in each tube

* Containing carrier RNA and internal control.

† Reconstituted in 6 ml protease solvent (PS).

‡ Presence of elution fluid (TOPE) is not monitored in the load check.

§ Only required for the QIAamp DSP 96 Virus MDx (AW1) protocol.

Protocol: Automated Isolation and Purification of Viral Nucleic Acids from Plasma and Serum

For isolation and purification of viral nucleic acids from 265 µl of EDTA- or citrate-treated plasma and serum using the BioRobot MDx DSP system.

Important points before starting

- Each sample must be labeled with a bar code, otherwise it will not be processed. The person with supervisor access to the QIAsoft MDx DSP software can select whether to use unique bar codes (the default setting) or nonunique bar codes. For details, refer to the *BioRobot MDx DSP User Manual*.

  ⓘ If using the nonunique bar codes option, the same bar code can be used to label different samples. Be careful to avoid mixup of samples containing the same bar code.

- Orient bar code labeled samples in the tube holders of the sample tracking system so that the bar codes face the bar code reader camera on the left side of the BioRobot MDx workstation. Bar code labels should be stuck to the sample tubes so that the bar code lines are horizontal. Bar code labels should be compatible with the BioRobot MDx workstation (for details, see the *BioRobot MDx DSP User Manual*). Each tube should contain at least 500 µl of sample.
- The tip disposal container must contain an empty, fully opened tip disposal bag. The drawer with the tip disposal container must be pushed back completely.
- The system liquid container, the waste container, and the vacuum trap must be positioned correctly in their respective holders and dry on the outside. The sensor of the system liquid container must be dry.
- If the BioRobot MDx DSP system develops an error (e.g., insufficient vacuum pressure) during automated sample preparation, follow the instructions in the display messages that appear or refer to the *BioRobot MDx DSP User Manual*. Display messages (which appear in English in the software) or their relevant translations are listed in Appendix A, page 39.

Things to do before starting

- Add carrier RNA reconstituted in elution buffer (AVE) and internal control to lysis buffer (AL), according to the instructions on page 26.
- Ensure that QIAGEN Protease (QP), wash buffer 1 (AW1), wash buffer 2 (AW2), and ethanol have been prepared according to the instructions in "Preparing reagents and buffers" on page 26.

Procedure

1. **Equilibrate samples to room temperature (15–25°C) and mix well by inverting several times.**

   ⓘ Cryoprecipitates formed during freeze–thawing may clog the membranes of the QIAamp 96 plate. If cryoprecipitates are visible, they should be pelleted by centrifugation at approximately 6800 x *g* for 3 min. The cleared supernatant should be aspirated and processed immediately without disturbing the pellet.

   ⓘ Avoid creating large bubbles, as they may interfere with the liquid detection system.

2. **Make sure that the BioRobot MDx workstation is switched on.**

   The power switch is located on the lower right of the front panel of the BioRobot MDx DSP workstation.

3. **Switch on the computer and monitor.**

4. **Launch the QIAsoft MDx DSP software.**

   The software can be started from the desktop.

5. **Login with your username and password.**

   The username and password for each user can be defined by the supervisor. For detailed information on how to assign a username and password to each user, refer to the *BioRobot MDx DSP User Manual*.

6. **Click  to activate the adjacent dialog field. Use the hand-held bar code reader to scan the Q-Card bar code into this field.**

   One bar code is printed on the Q-Card label for each protocol. After scanning the appropriate bar code, the protocol is automatically selected.

   ⓘ Make sure to choose the appropriate protocol. Choose the upper bar code for the QIAamp DSP 96 Virus MDx (AW1) protocol, with AW1 wash. Choose the lower bar code for the QIAamp DSP 96 Virus MDx protocol, without AW1 wash.

   The Q-Card bar code contains information about the material number, lot number, and expiration date of the reagents. This information, as well as which protocol was chosen, will be logged in a result file generated for every processed run.

7. **Click 

to start the QIAamp DSP 96 Virus MDx Protocol.**

8. **Choose whether to enter a user-specific ID for the protocol run.**

   If you choose this option, a dialog field will appear. Enter a specific ID using letters and numbers only (the ID must be unique and cannot be used again). At the end of the protocol run, the software will create result files

with this specific ID as the filename. The contents of these files will be the same as those for the default result files.

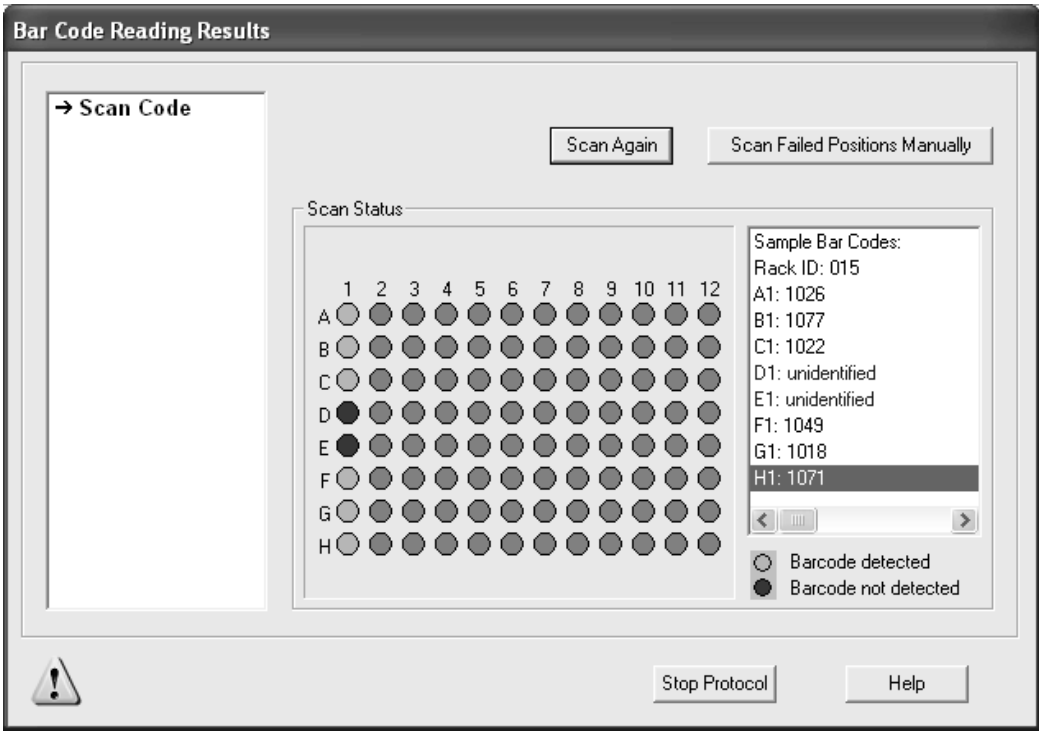
9. Follow the on-screen instructions for setting up the worktable.

For the remaining steps required to set up the BioRobot MDx workstation (see page 39), you can choose to be guided either by a series of display messages or by a reference table. We recommend that only experienced users choose the latter option.

ⓘ Carefully pour lysis buffer (AL) containing carrier RNA (and optional internal control) into the disposable trough (DT) in order to avoid creating large bubbles, which may interfere with the liquid detection system.

After the worktable hood is locked the BioRobot MDx DSP system checks if every component needed for the protocol is in place and if sample bar codes can be read. If an error is detected (e.g., a component is missing or the volume of a reagent is too low), the workstation pauses and a display message prompts you to recheck the cause of the error thoroughly. Follow the steps detailed in each display message before continuing.

If some bar codes could not be read, the following dialog box appears:



Unidentified samples are highlighted in red. After correcting the cause of the bar code reading error, either click "Scan Again" to scan all bar codes again, or click "Scan Failed Positions Manually" to scan only the bar codes

of the unidentified samples. If “Scan Failed Positions Manually” is clicked, the following dialog box appears:

Either scan each bar code using the hand-held bar code reader, or type in the number of each bar code. When typing in bar code numbers, each bar code number must be entered twice.

Once the protocol has passed the load check it will complete the purification process without interruptions.

10. The BioRobot MDx DSP system generates result files.

After the run is completed, two result files are generated to summarize all the data relevant to the run. The files are automatically saved in rich text format (*.rtf) and in comma separated values format (*.csv) under the default directory **C:\Program Files\QIAsoft MDx DSP\UserData\ReportData** . The identification bar code of the processed Elution Microtubes CL (EMT) is used as a filename. In addition, result files are saved under a user-specific filename if you chose this option at the start of the protocol: these files are saved under the same default directory. The content of all 4 files is the same (see example, page 50, and Table 14, page 51).

11. Clean up the BioRobot MDx workstation.

The software displays a series of messages that guide you through the steps of the cleanup procedure (see page 39).

Appendix A: Display Messages

The messages displayed by the software protocol during worktable setup and cleanup (steps 9 and 11) are listed below. The numbers of the messages listed below correspond to the numbers of the messages displayed by the software.

Note: Some of the display messages listed below may not appear during the protocol run (e.g., error messages).

Message type	Message text	Task/comments
Error	1. You scanned or entered the wrong Q-Card bar code. The protocol can only be stopped.	Make sure that you use the QIAamp DSP 96 Virus MDx Kit.
Error	2. The QIAGEN kit has exceeded the expiration date. The protocol can only be stopped.	You used an expired QIAamp DSP 96 Virus MDx Kit.
Guidance	3. Do you want to enter a specific run ID? A result file will be generated with the name of the entered run ID.	
Guidance	4. Please enter a specific run ID.	
Error	5. You did not enter a specific run ID. No separate result files with your run ID will be generated.	If you wanted to enter a specific run ID, stop the protocol and start again.
Guidance	6. Disconnect the tubing of the system liquid container and remove the lid. Refill the container with deionized water. Close the lid and reconnect the tubing.	
Error	7. The system liquid container sensor is defective or incorrectly adjusted. The protocol can only be stopped.	Try again with a full system liquid container, or contact QIAGEN Technical Services.

Message type	Message text	Task/comments
Guidance	8. Disconnect the tubing of the waste container and remove the lid. Empty the container. Close the lid and reconnect the tubing.	
Error	9. The waste container sensor is defective or incorrectly adjusted. The protocol can only be stopped.	Try again with an empty waste container, or contact QIAGEN Technical Services.
Guidance	10. Disconnect the tubing of the vacuum trap and the condensate trap. Remove the lid of the vacuum trap. Empty both traps. Close the vacuum trap lid and reconnect the tubing.	
Error	11. The vacuum trap sensor is defective or incorrectly adjusted. The protocol can only be stopped.	Try again with an empty vacuum trap, or contact QIAGEN Technical Services.
Guidance	12. Attach a new tip-disposal bag.	
Guidance	13. Do you want to be guided by the wizard to set up the worktable?	
Guidance	14. Reconstitute wash buffer 1 (AW1) and wash buffer 2 (AW2) according to the instruction on the bottle label. Fill the ethanol bottle (BOT) with 350 ml 100% ethanol, and add 350 µl reconstituted wash buffer 2 (AW2) (for a final 1/1000 dilution of AW2).	

Message type	Message text	Task/comments
Guidance	15. Place open bottles of wash buffer 1 (AW1), wash buffer 2 (AW2), and ethanol into compatible slots in the reagent carousel. Ensure that the bar codes face outward. Place the system liquid bottle filled with a minimum of 700 ml deionized water into the reagent carousel.	
Guidance	16. Place the black multiwell-plate holder onto the worktable. Place the black channeling adapter into this holder.	
Guidance	17. Place a silver multiwell-plate holder onto the worktable. Place a QIAamp 96 plate into this holder. Make sure well A1 is orientated to the left and the bar code faces toward the right.	
Guidance	18. Place a new S-Block onto the heat transfer adapter in the cooling and heating system. Make sure well A1 is orientated to the left.	
Guidance	19. Place the vacuum manifold lid onto the slot that is to the right of the vacuum manifold base. Place the vacuum manifold top above the vacuum manifold lid.	
Guidance	20. Pull the tip-tray drawer outwards. Place red tip trays with 1100 µl disposable tips into the red tip-tray holders 1, 2, 4–8.	

Message type	Message text	Task/comments
Guidance	21. Reconstitute QIAGEN Protease (QP) as described in the QIAGEN kit handbook. Mix carefully. Place the open bottle containing a minimum of 6 ml reconstituted QIAGEN Protease (QP) into the reagent holder for 1 bottle (QP).	
Guidance	22. Into the reagent holder for 2 troughs, load 2 disposable troughs (DT). Place the reagent holder into MP Slot 2. Ensure AL is on the left and EtOH is on the right.	
Guidance	23. Prepare a bottle of lysis buffer (AL) with carrier RNA and internal control (refer to the QIAGEN kit handbook). Carefully transfer the contents of the bottle into the trough at position AL in order to avoid creating large air bubbles.	
Guidance	24. Place 8 new microtubes each containing 2 ml elution buffer (AVE) into the reagent holder for microtubes and trough (position AVE). Remove the caps from the tubes.	
Guidance	25. Place 4 microtubes each containing 1.4 ml elution fluid (TOPE) into the reagent holder for microtubes and trough (position TOPE). Remove the caps from the tubes.	

Message type	Message text	Task/comments
Guidance	26. Place an Elution Microtubes CL (EMT) onto the elution microtube adapter. Place the assembly onto the reagent holder for microtubes and trough. Make sure the bar code faces toward the right.	
Guidance	27. Attention: The robotic arm moves to the right if you click "Next"!	
Guidance	28. Remove the sample protection shield. Place open tubes containing samples into the tube holders.	
Guidance	29. Load all 12 tube holders into the sample tracking system. Ensure the sample bar codes face towards the left. Replace the sample protection shield.	
Guidance	30. Summary table for worktable setup. Use the slider to move the robotic arm for easy access to all positions.	
Guidance	31. Close the worktable hood. Click "Continue" to start the load check.	
Error	32. The worktable hood is not closed. Slide the worktable hood down until it clicks.	Follow the instructions on the screen.
Error	33. The worktable hood is not closed or the worktable hood lock is defective. The protocol can only be stopped.	Try again with the worktable hood closed, or contact QIAGEN Technical Services.

Message type	Message text	Task/comments
Error	34. The reagent carousel door is not closed. Close the reagent carousel door.	Follow the instructions on the screen.
Error	35. The reagent carousel door is not closed or the reagent carousel door lock is defective. The protocol can only be stopped.	Try again with the rotor door closed, or contact QIAGEN Technical Services.
Error	36. The chosen protocol does not match the QIAamp 96 plate used. The protocol can only be stopped.	Make sure that you use the QIAamp DSP 96 Virus MDx Kit and choose the correct protocol.
Error	37. Place the system liquid bottle filled with a minimum of 700 ml deionized water into the reagent carousel.	Follow the instructions on the screen.
Error	38. Fill the ethanol bottle (BOT) with 350 ml ethanol (96–100%), and add 350 µl reconstituted wash buffer 2 (AW2) (for a final 1/1000 dilution of AW2).	Follow the instructions on the screen.
Error	39. Place a tip tray containing 1100 µl disposable tips into tip-tray holder 1.	Follow the instructions on the screen.
Error	40. Place a tip tray containing 1100 µl disposable tips into tip-tray holder 2.	Follow the instructions on the screen.
Error	41. Place a tip tray containing 1100 µl disposable tips into tip-tray holder 4.	Follow the instructions on the screen.
Error	42. Place a tip tray containing 1100 µl disposable tips into tip-tray holder 5.	Follow the instructions on the screen.

Message type	Message text	Task/comments
Error	43. Place a tip tray containing 1100 µl disposable tips into tip-tray holder 6.	Follow the instructions on the screen.
Error	44. Place a tip tray containing 1100 µl disposable tips into tip-tray holder 7.	Follow the instructions on the screen.
Error	45. Place a tip tray containing 1100 µl disposable tips into tip-tray holder 8.	Follow the instructions on the screen.
Error	46. Place an Elution Microtubes CL (EMT) with lid onto the elution microtube adapter. Then place the assembly onto MP Slot 3.	Follow the instructions on the screen.
Error	47. Make sure the bar code of the Elution Microtubes CL (EMT) faces toward the right.	Follow the instructions on the screen.
Error	48. Fill the wash buffer 1 (AW1) bottle in the reagent carousel with 164 ml buffer AW1. Place the bottle back into the reagent carousel and ensure that the bar code faces outward. Close the reagent carousel door properly.	Follow the instructions on the screen.
Error	49. Fill the wash buffer 2 (AW2) bottle in the reagent carousel with 191 ml buffer AW2. Place the bottle back into the reagent carousel, and ensure that the bar code faces outward. Close the reagent carousel door properly.	Follow the instructions on the screen.

Message type	Message text	Task/comments
Error	50. Place an empty disposable trough (DT) into the reagent holder for 2 troughs (position EtOH). Fill the trough with 10 ml ethanol.	Follow the instructions on the screen.
Error	51. The cooling and heating system failed to reach the required temperature. The protocol can only be stopped.	Try again or contact QIAGEN Technical Services.
Error	52. The high-speed dispensing system or the liquid detection system is defective and/or not adjusted within specifications. The protocol can only be stopped.	Try again or contact QIAGEN Technical Services.
Error	53. The vacuum manifold failed to reach the required temperature. The protocol can only be stopped.	Try again or contact QIAGEN Technical Services.
Error	54. The vacuum manifold failed to reach the required pressure. The protocol can only be stopped.	Try again or contact QIAGEN Technical Services.
Guidance	55. Sample preparation ended at *<Time (hh:mm)>*. Remove the Elution Microtubes CL (EMT) containing eluates from the reagent holder for microtubes and trough.	
Guidance	56. Discard the AVE and TOPE microtubes. Discard the QP bottle.	

Message type	Message text	Task/comments
Guidance	57. Discard the EtOH and AL troughs. Discard the tip trays. Discard the S-Block. Discard the QIAamp 96 plate.	
Guidance	58. Remove the channeling adapter, both multiwell-plate holders (black and silver), and the elution microtube adapter (blue). Submerge them all in disinfectant. Submerge the adapters/holders in deionized water. Dry with a lint free paper towel.	
Guidance	59. Remove the system liquid bottle from the reagent carousel and pour system liquid over the heating plate in the vacuum manifold base. Ensure that the plate is completely covered with system liquid.	
Guidance	60. Click "Continue" to remove system liquid from the vacuum manifold base. While liquid is leaving the vacuum manifold base, continue to empty the system liquid bottle over the heating plate.	
Guidance	61. Replace the caps on the bottles in the reagent carousel if no subsequent protocol run will be performed.	

Message type	Message text	Task/comments
Guidance	62. Attention: The robotic arm moves to the right if you click "Continue".	
Guidance	63. Discard the sample tubes.	
Guidance	64. Empty the tip-disposal bag.	
Guidance	65. Protocol finished! The result files are saved in the folder <name of folder>.	
Error	66. Wash buffer 1 (AW1) has expired. Remove the AW1 bottle from the reagent carousel and replace it with a new one. Ensure that the new bottle is open and the bar code faces outward. Close the reagent carousel door.	Follow the instructions on the screen.
Error	67. Wash buffer 2 (AW2) has expired. Remove the AW2 bottle from the reagent carousel and replace it with a new one. Ensure that the new bottle is open and the bar code faces outward. Close the reagent carousel door.	Follow the instructions on the screen.
Guidance	68. Reconstitute wash buffer 2 (AW2) according to the instruction on the bottle label. Fill the ethanol bottle (BOT) with a minimum of 350 ml ethanol (96–100%), and add 350 µl reconstituted wash buffer 2 (AW2) (for a final 1/1000 dilution of AW2).	

Message type	Message text	Task/comments
Guidance	69. Place open bottles of wash buffer 2 (AW2), and ethanol into compatible slots in the reagent carousel. Ensure that the bar codes face outward. Place the system liquid bottle filled with a minimum of 700 ml deionized water into the reagent carousel.	
Error	70. There are not enough 1100 µl disposable tips for this protocol run. Place red tip trays containing 1100 µl tips into red tip-tray holders 1, 2, 4–8.	

Appendix B: Result File*

Operator:
Date: YYYY MM DD
Start time: HH:MM:SS
Run time:
Kit name:
Number of samples processed:

Bar code of Elution Microtubes CL (EMT):
Operator-specified run ID:
Q-Card bar code:
Kit catalog number:
Kit lot number:
Kit expiration date: YYYY MM DD

BioRobot MDx ID:
QIAsoft MDx DSP version:
Protocol version:
Supervisor-selected bar code: Default/Bar code unique/Bar code nonunique†

Message log file name:
Report log file name:

Manually entered bar codes: no sample bar codes entered manually†
Technical process check: PASSED/FAILED†
Maintenance: Required maintenance tasks were not carried out before the protocol was run/Maintenance done†

Bar code and result table:

Position	Bar code	Result (1 = valid, 0 = invalid)
A1		
B1		
C1		
D1		
…	…	…
E12		
F12		
G12		
H12		

* The fields in this result file are empty. The QIAsoft MDx DSP Operating System fills in these fields at the end of the protocol run.

† The text that appears in this field depends on what happens during the protocol run.

Table 14. Explanation of Errors in Result File

Error	Possible cause
Sample marked as "invalid"	■ During aspiration of sample from the sample tube, the bar code could not be read. No sample was transferred to the S-Block. ■ During aspiration of sample from the sample tube, no sample was detected. ■ During aspiration of sample from the sample tube, a clot was detected. ■ Cryoprecipitates prevented transfer of the correct volume of sample from the sample tube to the S-Block. ■ After sample transfer, not enough sample was detected in the S-Block.
Row in the "Result" column is empty	A processing error occurred during sample preparation. The protocol was stopped.

Appendix C: Calculating the Amount of Internal Control

To monitor the efficiency of sample preparation and downstream assay, an internal control, provided with the diagnostic downstream assay, is added to the lysis buffer (AL). To calculate the amount of internal control needed in QIAamp DSP 96 Virus MDx protocols, the amount of lysis buffer (AL) and volume of the eluate must be taken into account.

Determining how much internal control will be in downstream reactions

To determine the volume of internal control that will be present in the downstream assay, use the formula:

$$IC_{RXN} = \frac{IC_{AL} \times AL_{SAM} \times EL_{RXN}}{(AL_{TOT} + IC_{AL}) \times EL_{SAM}}$$

where:

IC_{RXN} = Volume of internal control per downstream reaction

IC_{AL} = Volume of internal control added to lysis buffer (AL)

AL_{SAM} = Volume of lysis buffer (AL) per sample

EL_{RXN} = Volume of eluate per downstream reaction

AL_{TOT} = Total volume of lysis buffer (AL) used in the protocol

EL_{SAM} = Volume of eluate per sample

As an example, User 1 has added 300 µl of internal control solution (IC_{AL}) to 33 ml lysis buffer (AL_{TOT}). Using the standard procedure, there is 275 µl of lysis buffer per sample (AL_{SAM}), and elution volume is 83 µl (EL_{SAM}). User 1 uses 50 µl of eluate per downstream reaction (EL_{RXN}). The volume of internal control solution in each downstream reaction (IC_{RXN}) is:

$$IC_{RXN} = \frac{300\ \mu l \times 275\ \mu l \times 50\ \mu l}{(33{,}000\ \mu l + 300\ \mu l) \times 83\ \mu l} = 1.49\ \mu l$$

The final downstream reactions will contain 1.49 µl of internal control solution per reaction.

Determining how much internal control solution to add before starting

Alternatively, if you know the amount of internal control that you want to have present in the downstream assay (IC_{RXN}), then you need to determine the amount to add to the lysis buffer before starting purification (IC_{AL}). To calculate this value, use the formula:

$$IC_{AL} = \frac{IC_{RXN} \times AL_{TOT} \times EL_{SAM}}{(AL_{SAM} \times EL_{RXN}) - (IC_{RXN} \times EL_{SAM})}$$

As an example, User 2 is working with a diagnostic assay that is validated for use with 2.0 µl of internal control solution per reaction (IC_{RXN}) and 40 µl of eluate per reaction (EL_{RXN}). User 2 follows a protocol with 1000 µl of lysis buffer per sample (AL_{SAM}) and a 120 µl elution volume (EL_{SAM}). The amount of internal control solution (IC_{AL}) that User 2 needs to add to 33.0 ml lysis buffer (AL_{TOT}) is:

$$IC_{AL} = \frac{2.0\ \mu l \times 33{,}000\ \mu l \times 120\ \mu l}{(1000\ \mu l \times 40\ \mu l) - (2.0\ \mu l \times 120\ \mu l)} = 199\ \mu l$$

User 2 needs to add 199 µl of internal control solution to 33 ml lysis buffer (AL) before purification.

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